

**WHAT IS CLAIMED IS:**

1. A method for determining formaldehyde, which comprises bringing glutathione-dependent formaldehyde dehydrogenase having a ratio of reactivity with thio-NAD to reactivity  
5 with NAD of not less than 30%, glutathione and an oxidized coenzyme into contact with a sample, and analyzing a compound resulting from the enzymatic reaction.
2. The method of claim 1, wherein the ratio of reactivity  
10 with thio-NAD to reactivity with NAD of glutathione-dependent formaldehyde dehydrogenase is not less than 60%.
3. The method of claim 1, wherein the glutathione-dependent formaldehyde dehydrogenase has the following physico-  
15 chemical properties:  
action: production of S-formylglutathione and reduced coenzyme by action on formaldehyde in the presence of one oxidized coenzyme selected from the group consisting of NADs, NADPs, thio-NADs and thio-NADPs and reduced glutathione  
20 optimal pH: about 7.5-about 8.5  
pH stability: about 6.0-about 9.0, and  
heat stability: about 40°C or less (pH 7.5, 30 min).
4. The method of any of claim 1, wherein the glutathione-  
25 dependent formaldehyde dehydrogenase is derived from microorganism.
5. The method of claim 4, wherein the glutathione-dependent formaldehyde dehydrogenase is derived from methylotrophic  
30 yeast.
6. The method of claim 5, wherein the glutathione-dependent formaldehyde dehydrogenase is derived from *Hansenula* yeast.

7. The method of claim 6, wherein the glutathione-dependent formaldehyde dehydrogenase is derived from *Hansenula nonfermentans* IFO1473.

5 8. A method for determining formaldehyde, which comprises bringing glutathione-dependent formaldehyde dehydrogenase having a ratio of reactivity with thio-NAD to reactivity with NAD of not less than 30%, glutathione, one compound selected from the group consisting of thio-NADs and thio-  
10 NADPs, and one compound selected from the group consisting of reduced NADs and reduced NADPs into contact with a sample to allow cycling reaction and analyzing changes in the amount of a compound due to the reaction.

15 9. A method for determining formaldehyde, which comprises bringing glutathione-dependent formaldehyde dehydrogenase having a ratio of reactivity with thio-NAD to reactivity with NAD of not less than 30%, glutathione, one compound selected from the group consisting of reduced thio-NADs and  
20 reduced thio-NADPs, and one compound selected from the group consisting of NADs and NADPs into contact with a sample to allow cycling reaction and analyzing changes in the amount of a compound due to the reaction.

25 10. The method of claim 8, wherein the amount of the reduced thio-NADP or reduced thio-NAD compound is analyzed.

11. The method of any of claim 1, wherein a minimum detection limit of the formaldehyde is not more than 1  
30  $\mu\text{mol/L}$ .

12. The method of any of claim 8, wherein a minimum detection limit of the formaldehyde is not more than 1  
 $\mu\text{mol/L}$ .

13. A method for determining a biological component, which comprises, in the measurement of a biological component that produces formaldehyde as a reaction intermediate, measuring  
5 produced formaldehyde by the method of claim 1.

14. A method for determining a biological component, which comprises, in the measurement of a biological component that produces formaldehyde as a reaction intermediate, measuring  
10 produced formaldehyde by the method of claim 8.

15. A method for determining homocysteine, which comprises bringing betaine, betaine-homocysteine methyltransferase, dimethylglycine oxidase and, where necessary, sarcosine  
15 oxidase into contact with a sample, and measuring, according to the method of claim 1, formaldehyde produced by the enzymatic reactions.

16. A method for determining homocysteine, which comprises  
20 bringing betaine, betaine-homocysteine methyltransferase, dimethylglycine oxidase and, where necessary, sarcosine oxidase into contact with a sample, and measuring, according to the method of claim 8, formaldehyde produced by the enzymatic reactions.

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17. A method for determining creatine or creatinine, which comprises reacting creatine amidohydrolase, sarcosine oxidase, and, where necessary, creatinine amidohydrolase and measuring, according to the method of claim 1, formaldehyde  
30 produced by the enzymatic reactions.

18. A method for determining creatine or creatinine, which comprises reacting creatine amidohydrolase, sarcosine oxidase, and, where necessary, creatinine amidohydrolase and

measuring, according to the method of claim 8, formaldehyde produced by the enzymatic reactions.

19. A method for determining homocysteine, which comprises  
5 bringing transferase utilizing homocysteine and other compound as a pair of substrates and said other compound into contact with a sample and measuring the resulting compound.

10 20. The method of claim 19, wherein the transferase and said other compound is a combination selected from the group consisting of betaine-homocysteine methyltransferase and betaine, betaine-homocysteine methyltransferase and dimethylthetin, homocysteine methyltransferase and S-  
15 adenosylmethionine, and N5-methyltetrahydrofolate-homocysteine methyltransferase and N5-methyltetrahydrofolate, and the resulting compound is methionine.

21. The method of claim 19, wherein the betaine, betaine-  
20 homocysteine methyltransferase, dimethylglycine oxidase and, where necessary, sarcosine oxidase are brought into contact with a sample, hydrogen peroxide produced by the enzymatic reactions is reacted with hydrogen donor chromogenic reagent and, where necessary, coupler, in the presence of peroxidase,  
25 and the resulting pigment is measured.

22. The method of claims 21, wherein the dimethylglycine oxidase is an enzyme stable to thiol compound.

30 23. The method of claim 22, wherein the thiol compound is at least one kind selected from the group consisting of dithiothreitol, dithioerythritol, 2-mercaptoethanol, 2-mercaptoethanesulfonate, 2-mercaptoethylamine, cysteine, homocysteine, N-acetylcysteine, thioglycerol, thioglycolic

acid, reduced glutathione and salts thereof.

24. The method of claim 22, wherein the thiol compound is dithiothreitol.

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25. The method of claim 22, wherein the dimethylglycine oxidase shows an enzyme activity retained at least by 50% in the presence of 0.05 mmol/L dithiothreitol relative to the enzyme activity in the absence of dithiothreitol.

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26. The method of claim 22, wherein the dimethylglycine oxidase is an enzyme having the following physico-chemical properties:

action: acting on dimethylglycine in the presence of oxygen  
15 to produce sarcosine, formaldehyde and hydrogen peroxide,  
and

K<sub>m</sub> value for dimethylglycine: not more than 15 mM.

27. The method of claim 22, wherein the dimethylglycine  
20 oxidase is derived from a microorganism.

28. The method of claim 27, wherein the dimethylglycine oxidase is derived from a microorganism belonging to the genus *Arthrobacter* or the genus *Streptomyces*.

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29. The method of claim 28, wherein the dimethylglycine oxidase is derived from *Arthrobacter nicotianae* IF014234 or *Streptomyces mutabilis* IF012800.

30 30. The method of claim 19, wherein the betaine, betaine-homocysteine methyltransferase, dimethylglycine oxidase and, where necessary, sarcosine oxidase are brought into contact with a sample, formaldehyde produced by the enzymatic reactions is reacted with formaldehyde dehydrogenase and

oxidized coenzyme and the resulting reduced coenzyme is measured.

31. The method of claim 30, wherein the dimethylglycine  
5 oxidase is an enzyme stable to thiol compound.

32. The method of claim 31, wherein the thiol compound is  
at least one kind selected from the group consisting of  
dithiothreitol, dithioerythritol, 2-mercaptoethanol, 2-  
10 mercaptoethanesulfonate, 2-mercaptoethylamine, cysteine,  
homocysteine, N-acetylcysteine, thioglycerol, thioglycolic  
acid, reduced glutathione and salts thereof.

33. The method of claim 31, wherein the thiol compound is  
15 dithiothreitol.

34. The method of claim 31, wherein the dimethylglycine  
oxidase shows an enzyme activity retained at least by 50% in  
the presence of 0.05 mmol/L dithiothreitol relative to the  
20 enzyme activity in the absence of dithiothreitol.

35. The method of claim 31, wherein the dimethylglycine  
oxidase is an enzyme having the following physico-chemical  
properties:  
25 action: acting on dimethylglycine in the presence of oxygen  
to produce sarcosine, formaldehyde and hydrogen peroxide,  
and  
Km value for dimethylglycine: not more than 15 mM.

30 36. The method of claim 31, wherein the dimethylglycine  
oxidase is derived from a microorganism.

37. The method of claim 36, wherein the dimethylglycine  
oxidase is derived from a microorganism belonging to the

genus *Arthrobacter* or the genus *Streptomyces*.

38. The method of claim 37, wherein the dimethylglycine oxidase is derived from *Arthrobacter nicotianae* IF014234 or  
5 *Streptomyces mutabilis* IF012800.

39. The method of claim 19, wherein the betaine, betaine-homocysteine methyltransferase, dimethylglycine oxidase and, where necessary, sarcosine oxidase are brought into contact  
10 with a sample, formaldehyde produced by the enzymatic reactions is reacted with glutathione, glutathione-dependent formaldehyde dehydrogenase and oxidized coenzyme and the resulting reduced coenzyme is measured.

15 40. The method of claim 39, wherein the dimethylglycine oxidase is an enzyme stable to thiol compound.

41. The method of claim 40, wherein the thiol compound is at least one kind selected from the group consisting of  
20 dithiothreitol, dithioerythritol, 2-mercaptoethanol, 2-mercaptoethanesulfonate, 2-mercaptoethylamine, cysteine, homocysteine, N-acetylcysteine, thioglycerol, thioglycolic acid, reduced glutathione and salts thereof.

25 42. The method of claim 40, wherein the thiol compound is dithiothreitol.

43. The method of claim 40, wherein the dimethylglycine oxidase shows an enzyme activity retained at least by 50% in  
30 the presence of 0.05 mmol/L dithiothreitol relative to the enzyme activity in the absence of dithiothreitol.

44. The method of claim 40, wherein the dimethylglycine oxidase is an enzyme having the following physico-chemical

properties:

action: acting on dimethylglycine in the presence of oxygen to produce sarcosine, formaldehyde and hydrogen peroxide, and

5 Km value for dimethylglycine: not more than 15 mM.

45. The method of claim 40, wherein the dimethylglycine oxidase is derived from a microorganism.

10 46. The method of claim 45, wherein the dimethylglycine oxidase is derived from a microorganism belonging to the genus *Arthrobacter* or the genus *Streptomyces*.

47. The method of claim 46, wherein the dimethylglycine  
15 oxidase is derived from *Arthrobacter nicotianae* IF014234 or *Streptomyces mutabilis* IF012800.

48. The method of claim 40, wherein a minimum detection limit of the homocysteine is not more than 1  $\mu\text{mol/L}$ .

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49. Glutathione-dependent formaldehyde dehydrogenase having the following physico-chemical properties:

action: acting on formaldehyde in the presence of one coenzyme selected from the group consisting of NADs, NADPs,  
25 thio-NADs and thio-NADPs, reduced glutathione to produce S-formylglutathione, a reduced coenzyme,  
a ratio of reactivity with thio-NAD to reactivity with NAD: not less than 30%,  
optimal pH: about 7.5-about 8.5,  
30 pH stability: about 6.0-about 9.0, and  
heat stability: about 40°C or less (pH 7.5, 30 min).

50. The glutathione-dependent formaldehyde dehydrogenase of claim 49, which is derived from a microorganism.



51. The glutathione-dependent formaldehyde dehydrogenase of claim 50, which is derived from methylotrophic yeast.

5 52. The glutathione-dependent formaldehyde dehydrogenase of claim 51, which is derived from *Hansenula* yeast.

53. The glutathione-dependent formaldehyde dehydrogenase of claim 52, which is derived from *Hansenula nonfermentans*  
10 IF01473.

54. Dimethylglycine oxidase having the following physico-chemical properties:

action: acting on dimethylglycine in the presence of oxygen  
15 to produce sarcosine, formaldehyde and hydrogen peroxide, an enzyme activity is retained at least by 50% in the presence of 0.05 mmol/L dithiothreitol relative to the enzyme activity in the absence of dithiothreitol, and Km value for dimethylglycine: not more than 15 mM.

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55. The dimethylglycine oxidase of claim 54, which is derived from a microorganism.

56. The dimethylglycine oxidase of claim 55, which is  
25 derived from a microorganism belonging to the genus *Arthrobacter* or the genus *Streptomyces*.

57. The dimethylglycine oxidase of claim 56, which is derived from *Arthrobacter nicotianae* IF014234, or  
30 *Streptomyces mutabilis* IF012800.

58. A reagent kit for formaldehyde determination, which comprises at least buffer, glutathione, glutathione-dependent formaldehyde dehydrogenase having a ratio of

reactivity with thio-NAD to reactivity with NAD of not less than 30% and a reagent for analyzing a compound produced by the enzymatic reaction.

5 59. The reagent kit for formaldehyde determination of claim 58, wherein glutathione-dependent formaldehyde dehydrogenase has the following physico-chemical properties:  
action: production of S-formylglutathione and reduced  
coenzyme by action on formaldehyde in the presence of one  
10 oxidized coenzyme selected from the group consisting of NADs, NADPs, thio-NADs and thio-NADPs and reduced glutathione  
optimal pH: about 7.5-about 8.5  
pH stability: about 6.0-about 9.0, and  
heat stability: about 40°C or less (pH 7.5, 30 min).

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60. A reagent kit for formaldehyde determination, which comprises at least buffer, glutathione, glutathione-dependent formaldehyde dehydrogenase having a ratio of reactivity with thio-NAD to reactivity with NAD of not less  
20 than 30%, one compound selected from the group consisting of thio-NADs and thio-NADPs, and one compound selected from the group consisting of reduced NADs and reduced NADPs.

61. The reagent kit for formaldehyde determination of claim  
25 60, wherein the glutathione-dependent formaldehyde dehydrogenase has the following physico-chemical properties:  
action: production of S-formylglutathione and reduced  
coenzyme by action on formaldehyde in the presence of one  
oxidized coenzyme selected from the group consisting of NADs,  
30 NADPs, thio-NADs and thio-NADPs and reduced glutathione  
optimal pH: about 7.5-about 8.5  
pH stability: about 6.0-about 9.0, and  
heat stability: about 40°C or less (pH 7.5, 30 min).

62. A reagent kit for formaldehyde determination, which comprises at least buffer, glutathione, glutathione-dependent formaldehyde dehydrogenase having a ratio of reactivity with thio-NAD to reactivity with NAD of not less than 30%, one compound selected from the group consisting of reduced thio-NADs and reduced thio-NADPs, and one compound selected from the group consisting of NADs and NADPs.

63. The reagent kit for formaldehyde determination of claim 62, wherein the glutathione-dependent formaldehyde dehydrogenase has the following physico-chemical properties: action: production of S-formylglutathione and reduced coenzyme by action on formaldehyde in the presence of one oxidized coenzyme selected from the group consisting of NADs, NADPs, thio-NADs and thio-NADPs and reduced glutathione optimal pH: about 7.5-about 8.5 pH stability: about 6.0-about 9.0, and heat stability: about 40°C or less (pH 7.5, 30 min).

64. A reagent kit for homocysteine determination, which comprises, in addition to the reagent of claim 58, betaine, betaine-homocysteine methyltransferase, dimethylglycine oxidase and, where necessary, sarcosine oxidase.

65. A reagent kit for homocysteine determination, which comprises, in addition to the reagent of claim 60, betaine, betaine-homocysteine methyltransferase, dimethylglycine oxidase and, where necessary, sarcosine oxidase.

66. A reagent kit for homocysteine determination, which comprises, in addition to the reagent of claim 62, betaine, betaine-homocysteine methyltransferase, dimethylglycine oxidase and, where necessary, sarcosine oxidase.

67. A reagent kit for creatinine or creatine determination, which comprises, in addition to the reagent of claim 58, creatine amidinohydrolase, sarcosine oxidase and, where necessary, creatinine amidohydrolase.

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68. A reagent kit for creatinine or creatine determination, which comprises, in addition to the reagent of claim 60, creatine amidinohydrolase, sarcosine oxidase and, where necessary, creatinine amidohydrolase.

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69. A reagent kit for creatinine or creatine determination, which comprises, in addition to the reagent of claim 62, creatine amidinohydrolase, sarcosine oxidase and, where necessary, creatinine amidohydrolase.

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70. A reagent kit for homocysteine determination, which comprises at least buffer, transferase utilizing homocysteine and other compound as a pair of substrates, said other compound, and a reagent for analyzing a compound  
20 produced by the enzymatic reaction.

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71. The reagent kit for homocysteine determination of claim 70, wherein the transferase and said other compound are a combination selected from the group consisting of betaine-  
25 homocysteine methyltransferase and betaine, betaine-homocysteine methyltransferase and dimethylthetin, homocysteine methyltransferase and S-adenosylmethionine and N5-methyltetrahydrofolate-homocysteine methyltransferase and N5-methyltetrahydrofolate, and the produced compound is  
30 methionine.

72. A reagent kit for homocysteine determination, which comprises buffer, betaine, betaine-homocysteine methyltransferase, dimethylglycine oxidase and, where

necessary, sarcosine oxidase, and a reagent for measuring hydrogen peroxide produced by the enzymatic reactions.

73. The reagent kit of claim 72, wherein the reagent for  
5 measuring hydrogen peroxide comprises peroxidase, hydrogen donor chromogenic reagent and, where necessary, coupler.

74. The reagent kit of claim 72, wherein the  
dimethylglycine oxidase has the following physico-chemical  
10 properties:  
action: acting on dimethylglycine in the presence of oxygen to produce sarcosine, formaldehyde and hydrogen peroxide, an enzyme activity is retained at least by 50% in the presence of 0.05 mmol/L dithiothreitol relative to the  
15 enzyme activity in the absence of dithiothreitol, and  
Km value for dimethylglycine: not more than 15 mM.

75. A reagent kit for homocysteine determination, which comprises betaine, betaine-homocysteine methyltransferase,  
20 dimethylglycine oxidase and, where necessary, sarcosine oxidase, and a reagent for determination of formaldehyde produced by the enzymatic reactions.

76. The reagent kit of claim 75, which comprises  
25 formaldehyde dehydrogenase and oxidized coenzyme as reagents for determination of the formaldehyde.

77. The reagent kit of claim 75, which comprises glutathione, glutathione-dependent formaldehyde  
30 dehydrogenase and oxidized coenzyme as reagents for determination of the formaldehyde.

78. The reagent kit of claim 75, wherein the dimethylglycine oxidase has the following physico-chemical

properties:

action: acting on dimethylglycine in the presence of oxygen to produce sarcosine, formaldehyde and hydrogen peroxide, an enzyme activity is retained at least by 50% in the presence of 0.05 mmol/L dithiothreitol relative to the enzyme activity in the absence of dithiothreitol, and Km value for dimethylglycine: not more than 15 mM.